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Supplementary Selenium Influences the Response to Fatty Acid-Induced Oxidative Stress in Humans

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ABSTRACT

The mutual influences of wheat selenium (Se) and *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) on plasma Se and indicators of increased oxidative stress were investigated in a randomized, double-blind study with 31 women (23.5 ± 3.4 yr). Groups 1 and 2 ingested 5.4 g *n*-3 PUFA daily (as ethyl esters), whereas groups 3 and 4 received placebo capsules. Groups 2 and 3 received 3 slices of high-Se bread daily, providing 115 μg Se, in addition to the 77 ± 26 μg Se in the diet. Groups 1 and 4 received placebo slices. Blood samples were drawn at baseline and at 3 and 6 wk.

Serum Se concentrations increased in both groups given Se-enriched bread, but significantly less in subjects given *n*-3 PUFA (group 2). There were no changes in the plasma ratio α-tocopherol:mg cholesterol or plasma ascorbic acid levels. In group 1, plasma-conjugated dienes and thiobarbituric acid-reactive substances (TBARS) rose by 130% ($p < 0.005$) and 126% ($p < 0.005$), respectively. Two-way ANOVA showed significant interaction effects of Se and *n*-3 PUFA on changes in conjugated dienes ($p = 0.03$) and TBARS ($p = 0.015$), Se

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treatment apparently modifying the peroxidative effects of *n*-3 PUFA. In subjects receiving *n*-3 PUFA, changes in conjugated dienes and TBARS were negatively correlated with changes in serum Se. In summary, *n*-3 PUFA modified the effect of Se supplementation, whereas Se seemed to modify the peroxidative effects of *n*-3 PUFA.

Index Entries: Selenium; *n*-3 ethyl esters; lipid peroxides; humans; α-tocopherol; ascorbic acid.

INTRODUCTION

Epidemiological and experimental studies suggest that regular consumption of fish tends to lower the incidence of morbidity and mortality from heart diseases *(1–3)*. The beneficial effects have been attributed to the high content of polyunsaturated fatty acids (PUFA) in fish, although other fish components also have been suggested as favorable, among them selenium (Se). Fish Se concentrations are high, generally 0.2–0.5 mg/kg, and very low blood Se concentrations have been associated with increased risk of coronary heart disease *(4)*. Several explanations for these observations have been proposed, including Se's participation in the antioxidant defense system, and influence on platelet function and thromboxane/prostacyclin balance *(5,6)*.

Although the majority of studies indicate beneficial effects of a fish diet and fish oil supplements, the issue is, however, still controversial. PUFA are easily oxidized, and may at high levels of intake reduce tissue α-tocopherol concentrations in animals *(7,8)* and in humans *(9–11)*. Usually, several compounds in the antioxidant defense system will be involved when the oxidative stress of the organism increases, among them glutathione peroxidase (GSH-Px), in which Se is a cofactor. There is some evidence that an increased PUFA intake may influence Se status similar to the way in which it influences α-tocopherol, but this issue has so far only been studied in subjects with relatively low Se status *(12,13)*. An Se-PUFA interaction may also provide a partial explanation for the somewhat contradictory results from fish Se bioavailability studies *(14–17)*.

The present study was undertaken to investigate the influences of wheat Se and *n*-3 PUFA on plasma Se and indicators of increased oxidative stress in healthy, Norwegian women.

SUBJECTS AND METHODS

Subjects

Thirty-two female nutrition students volunteered to participate in the study. They had not taken any vitamin, mineral-, or fatty acid-containing supplements within 3 mo before the start of the study. They were all healthy, and none were pregnant, lactating, or using any type of

Table 1
Characteristics of the Study Population at Entry*

Age (years)	23.5 ± 3.4
Body Mass Index (kg/m^2)	21.0 ± 2.8
Serum Se (μmol/L)	1.40 ± 0.1
Serum total cholesterol (mmol/L)	4.3 ± 0.8
Serum HDL-cholesterol (mmol/L)	1.1 ± 0.2
Serum LDL cholesterol (mmol/L)	2.8 ± 0.7
Plasma triglycerides (mmol/L)	0.7 ± 0.3
Plasma α-tocopherol (μmol/L)	20.1 ± 3.1
Plasma ascorbic acid (μmol/L)	64.3 ± 11.0

*All values are mean ± SD. There were no significant differences between the groups initially (Duncan's multiple-range test, $p < 0.05$).

medication. Two of the participants smoked cigarets. One participant withdrew from the study midway for private reasons and was not substituted. Their mean (±SD) age, body mass index, initial serum Se, serum total, HDL, and LDL cholesterol, plasma triglycerides, plasma α-tocopherol, and initial ascorbic acid concentrations are shown in Table 1. The participants were living at home, and encouraged to maintain their usual daily routines and dietary habits. All participants gave informed consent, and the study protocol was approved by the Regional Ethics Committee of Medical Research.

Experimental Design

This study was randomized and double-blind. The intervention period lasted for 6 wk. The subjects were allocated to four groups. Group 1 (n = 8) consumed n-3 ethyl ester capsules and placebo bread. Group 2 (n = 8) consumed n-3 ethyl ester capsules and slices of bread providing 115 μg Se extra/d. Group 3 (n = 7) consumed placebo oil capsules and Se slices as group 2. Group 4 (n = 8), the control group, consumed placebo oil capsules and placebo bread during the study.

Diet and Dietary Supplements

Whole-grain wheat containing 7.9 mg Se/kg on analysis was obtained from R. Marts, Bonesteel, SD. The wheat was ground, mixed with appropriate amounts of locally grown Norwegian flour, and baked into bread giving 115 μg Se/90 g of bread (3 slices). The placebo bread was baked with the locally grown Norwegian flour exclusively.

n-3 Ethyl ester and placebo capsules were provided by J. C. Martens A/S, Oslo, Norway. Each *n*-3 PUFA capsule (EPAX 600 EE) contained 1 g ethyl esters of *n*-3 PUFA. The participants ate 9 capsules/d, dividing the intake between each of the three main meals of the day. The *n*-3 PUFA capsules provided totally 5.4 g EPA and DHA/d. The placebo capsules contained a mixture of fatty acids comparable to that of an ordinary Norwegian diet. The *n*-3 PUFA capsules contained 2 mg and the placebo capsules 0.5 mg mixed tocopherols/capsule, 20% of each amount being α-tocopherol. Their appearance was identical to the *n*-3 PUFA capsules. In both types of capsules, the ratio α-tocopherol:PUFA was 0.5.

Recent studies have shown that *n*-3 fatty acids given as ethyl esters are equally well absorbed and incorporated into blood lipids as free fatty acids or triglyceride fatty acids *(18–20)* and have similar inhibitory potential regarding platelet aggregation and thromboxane B_2 production in whole blood *(21)*.

The 32 participants collected duplicate portions of all food and liquid intake for four consecutive days in week three or four of the study. Weighed food records were obtained simultaneously.

In addition to chemical analysis of nutrient content in the duplicate portions, the content of dietary energy, fatty acid, and nutrients was calculated according to Norwegian Tables of Food Composition *(22)* by the data program "Fiber" (T. A. Ydersbond/Norwegian Dietetic Association, Oslo).

Blood Sampling

Blood samples, drawn by venipuncture in the morning after a 12-h fast, were taken from the participants at weeks –1, 0, 3, and 6. A total of 35 mL blood were drawn from the subjects each time. Samples for trace element analysis were drawn into evacuated tubes without additives manufactured for trace element analyses . Samples for blood lipid analyses, and plasma and erythrocyte α-tocopherol were drawn into EDTA-containing tubes. Samples for plasma ascorbic acid analysis were drawn into heparin-containing tubes. Samples for analysis of TBARS and conjugated dienes were drawn into plain tubes. All tubes were from Becton Dickinson Vacutainer Systems Europe.

Chemical Analysis

The duplicate diet portions were stored at –20°C until analysis. They were carefully thawed, homogenized, and partly analyzed as such (fatty acids, protein), and partly freeze-dried before analysis of elements.

For fatty acid analyses, samples of food homogenates and plasma were extracted, saponified, and the fatty acids esterified in 12% BF_3 in methanol *(23)*. The methyl esters were separated using a Carlo Erba 2900 gas chromatograph ("on-column" injection), equipped with a 50-m CP-

sil 88 (Chrompack) fused silica capillary column (id: 0.32 mm). The fatty acid composition was calculated using a Maxima 820 (Chromatography Workstation, installed in an IBM-AT), connected to the gas–liquid chromatograph and identification ascertained by standard mixtures of methylesters (Nu-Chek, Elysian, MN) *(24)*.

Serum triglycerides, HDL, and total cholesterol were determined spectrophotometrically using a Hitachi 704 Automatic Analyzer with reagents from Boehringer Mannheim (Germany). LDL cholesterol was calculated from total and HDL cholesterol according to Friedewald et al. *(25)*.

The plasma samples for α-tocopherol analysis were saponified and extracted twice with *n*-hexane. The HPLC determination of α-tocopherol was performed with a Shimadzu (LC-9A) pump, and a fluorescence detector (Shimadzu, RF-530, emission: 331 nm and excitation: 289 nm). The samples were injected by an autoinjector (Shimadzu, SIL-6B/9A), a column (4.6 × 150 mm) packed with silica gel (Shandor Hypersil H3) was used, and 2% 2-propanol in *n*-hexane (v/v) as mobile phase *(26)*.

Plasma ascorbic acid was analyzed immediately after sampling by HPLC and electrochemical detection according to the procedure described by Wang et al. *(27)*.

Conjugated dienes were determined after the following procedure: serum lipids were extracted according to Folch et al. *(28)*. Lipids were redissolved in cyclohexane and the absorbance at 234 nm measured (Hitachi spectrophotometer model 100-20) *(29)*. Both intra- and interassay coefficients of variation were 9%. TBARS were measured as follows: 1 mL serum was mixed with 2 mL TCA (10%) and centrifuged for 4 min (5000*g*). Equal amounts (2 mL) of supernatant and 1% TBA were mixed, and then incubated in a boiling water bath for 10 min. The absorbance at 535 nm was measured (Hitachi spectrophotometer model 100-20).

Se concentrations in samples of diet, serum, and urine were analyzed by atomic absorption spectrometry (Perkin Elmer 5000) with nickel as a matrix modifier as described by Maage et al. *(30)*. Before analysis, the samples were digested by addition of 65% nitric acid (Suprapure, Merck) and digested in a microwave oven (CEM, MSD-81D). The detection limit of the Se analysis was determined to be 0.44 μg/L, corresponding to 0.06 mg/kg in a dry biological sample by the digestion procedure used. The coefficient of variation (%) was tested by a certified standard material and found to be 3.5%.

Statistical Analyses

The data analysis was carried out using the SPSS-PC+ (SPSS-PC + Inc, Chicago, IL) statistical program package. $P < 0.05$ was considered statistically significant (two-sided tests).

Normality was checked by normal plots and the Kolmogorov-Smirnov test. Analysis of covariance was applied to correct for possible

confounding factors. Multiple regression analyses were performed to assess possible effects of sets of explanatory variables.

Two-way ANOVA was applied to test for individual and joint effects of *n*-3 PUFA and Se supplementation. Duncan's multiple-range test was used for comparing $n > 2$ group means. Changes within groups were tested for significance by paired *t*-tests. The Mann-Whitney test was employed to compare two groups.

RESULTS

Subject compliance, assessed by questionnaire and plasma fatty acid changes, was excellent throughout the study. Two participants complained about having a greasier skin in the intervention period. Body weight was monitored at the start and end of the study, and did not change.

Analysis of the 4-d duplicate portions indicates that the average daily intake of Se in the intervention period was 60–70 and 175–185 μg/d for the non-Se-bread and Se-enriched bread groups, respectively (Table 2). The dietary intake of α-tocopherol and PUFA was comparable in the four groups when disregarding the supplements. Table 2 also shows the α-tocopherol:PUFA ratio of the diet.

There were no significant changes in total plasma cholesterol or triglyceride concentrations in any group during the intervention. HDL cholesterol rose by 12 % ($p < 0.05$) in group 3 (placebo capsules + wheat Se), whereas LDL decreased by 8 % ($p < 0.05$) in group 2 (*n*-3 + wheat Se).

There was an expected and highly significant increase in plasma *n*-3 PUFA in the two *n*-3 PUFA groups, whereas oleic acid (18:1 *n*-9), linoleic (18:2 *n*-6), and dihomo-γ-linolenic acid (20:3 *n*-6) concentrations were reduced (Table 3). Plasma arachidonic acid (20:4 *n*-6) concentrations remained unchanged during the study.

The ratio of plasma (20:5 *n*-3):(20:4 *n*-6) ("the eicosanoid potential") increased three- to fourfold in the two *n*-3 PUFA groups ($p < 0.001$). In group 3 (placebo capsules + wheat Se), plasma EPA increased by 34 % (NS), in spite of a somewhat lower dietary EPA intake as compared to the control group. This resulted in an increase in eicosanoid potential from 0.27–0.36, which was significantly different from the control group ($p < 0.04$).

There was a significant increase in serum Se concentrations in both wheat Se groups (Fig. 1), although the increase in group 3 (placebo capsules) was significantly higher ($p < 0.05$) than in group 2. Both placebo bread groups experienced a decrease in serum Se concentrations of 7% (group 1) and 12 % (group 4), the latter being significant ($p < 0.03$).

Plasma α-tocopherol concentrations were very strongly correlated with total cholesterol concentrations through the study (at the end, $r = 0.87$, $p < 0.001$, $n = 31$), and when expressed per unit of cholesterol, no changes in plasma α-tocopherol were significant. Because of this strong

Table 2
Dietary Content of Se, Vitamin E, and PUFA as Analyzed by a 4-D Duplicate Portion Sampling*

	Selenium (μg/d)	α-tocopherol (mg/d)	PUFA (g/d)	Ratio α-toc:PUFA
Group 1	57 ± 23	6.6 ± 2.5	11.2 ± 3.0	0.6
Group 2	174 ± 14	5.6 ± 0.9	10.5 ± 3.6	0.5
Group 3	185 ± 27	4.6 ± 1.0	9.5 ± 2.5	0.5
Group 4	72 ± 21	6.4 ± 1.2	10.2 ± 2.9	0.6

*All values are mean ± SD. Apart from the Se intake, there were no significant differences between the groups.

correlation, α-tocopherol per mg cholesterol was used as a tocopherol measure in all statistical analyses.

The ratio plasma α-tocopherol:PUFA decreased only in group 1 (*n*-3 + placebo bread) ($p < 0.05$) during the study, whereas the ratio plasma α-tocopherol:(EPA + DHA) decreased significantly in both *n*-3 PUFA groups ($p < 0.01$).

There were no changes in plasma ascorbic acid concentrations in any group during the intervention period, nor did the concentration seem to be related to any of the parameters involved with lipid peroxidation.

There was a significant positive correlation between final concentrations of TBARS and conjugated dienes ($r = 0.76$, $p < 0.0005$), as well as changes in these parameters ($r = 0.55$, $p < 0.003$).

Changes in plasma conjugated dienes and TBARS, adjusted for initial values, are shown in Table 4. When analyzed by Duncan's multiple-range test, changes in TBARS were significantly higher ($p < 0.05$) in group 1 (*n*-3 PUFA + placebo bread) than in the other three groups. Two-way ANOVA showed significant interaction effects of Se and PUFA on conjugated dienes ($p = 0.03$) and TBARS ($p = 0.015$). There was also an effect of Se alone on TBARS ($p < 0.03$, negative), and of *n*-3 PUFA on conjugated dienes ($p = 0.002$) and TBARS ($p < 0.0005$) (positive).

No other interaction effects of Se and *n*-3 PUFA were significant by two-way ANOVA, nor were there any other effects of Se alone. Apart from the fatty acids and peroxidation measures, the only effect of *n*-3 PUFA significant by two-way ANOVA was a slight reduction in plasma triglycerides ($p = 0.05$). Plasma lipoproteins (HDL and LDL concentrations) were not affected by *n*-3 PUFA.

There was a strong, positive correlation between final plasma TBARS vs plasma *n*-3 fatty acids ($r = 0.53$, $p < 0.001$) (Fig. 2). In addition to the effects of Se treatment occurring in two-way ANOVA, there were correlations between changes in serum Se and plasma TBARS ($r = -0.78$, $p < 0.002$) and conjugated dienes ($r = -0.60$, $p < 0.03$) in the *n*-3-treated groups (Fig. 3). In groups 2 and 3, both receiving Se-enriched bread, there was a strong inverse relationship between changes in serum Se and plasma *n*-3 fatty acid concentrations ($r = -0.76$, $p < 0.002$, $n = 14$).

Table 3
Plasma Fatty Acid Composition Before and at the End of the 6-Wk Supplementation Period*

		Group 1: (n-3 capsules + placebo bread) n = 8	Group 2: (n-3 capsules+ wheat Se) n = 8	Group 3: (Placebo capsules + wheat Se) n = 7	Group 4: (Placebo capsules + placebo bread) n = 8
18:1n-9					
	Initial:	18.1 ± 3.2	17.7 ± 1.1	19.6 ± 2.8	18.0 ± 2.6
	Final:	16.5 ± 2.9	16.3 ± 2.0	19.8 ± 3.3	18.4 ± 2.6
	Change:	p<0.05	p=0.05	(NS)	(NS)
18:2n-6					
	Initial:	35.0 ± 2.4	32.9 ± 3.1	31.7 ± 3.9	33.3 ± 6.7
	Final:	29.1 ± 3.5	28.6 ± 3.6	31.7 ± 4.8	34.8 ± 4.2
	Change:	p<0.001	p<0.003	(NS)	(NS)
20:3n-6 (DGLA)					
	Initial:	1.0 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	1.3 ± 0.3
	Final:	0.6 ± 0.08	0.6 ± 0.1	1.3 ± 0.4	1.5 ± 0.2
	Change:	p<0.001	p<0.001	(NS)	p<0.04
20:4n-6					
	Initial:	5 ± 0.6	5.2 ± 1	5.6 ± 1.2	6.4 ± 1.3
	Final:	4.9 ± 0.6	4.7 ± 0.7	5.3 ± 0.9	6.0 ± 1.2
	Change:	(NS)	(NS)	(NS)	(NS)

20:5n-3 (EPA)	2.0 ± 1.4	2.4 ± 1.6	1.3 ± 0.7	1.6 ± 0.9
Initial:	8.6 ± 2.1	8.5 ± 1.9	1.8 ± 1	1.3 ± 0.3
Final:				
Change:	p<0.001	p<0.001	(NS)	(NS)
22:5n-3				
Initial:	0.57 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1
Final:	1.21 ±0.2	1.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Change:	p<0.001	p<0.001	(NS)	(NS)
22:6n-3 (DHA)				
Initial:	3.7 ± 0.8	4.1 ± 1.6	3.2 ± 0.7	3.2 ± 1.0
Final:	5.6 ± 0.9	5.9 ± 1	3.4 ± 0.9	3.2 ± 0.5
Change:	p<0.001	p<0.001	(NS)	(NS)
Total n-3				
Initial:	6.2 ± 2.2	7.1 ± 3.3	5.0 ± 1.4	5.4 ± 1.9
Final:	15.4 ± 3.1	15.8 ± 2.8	5.8 ± 2.1	5.1 ± 1.0
Change:	p<0.001	p<0.001	(NS)	(NS)

*All values are mean ± SD.

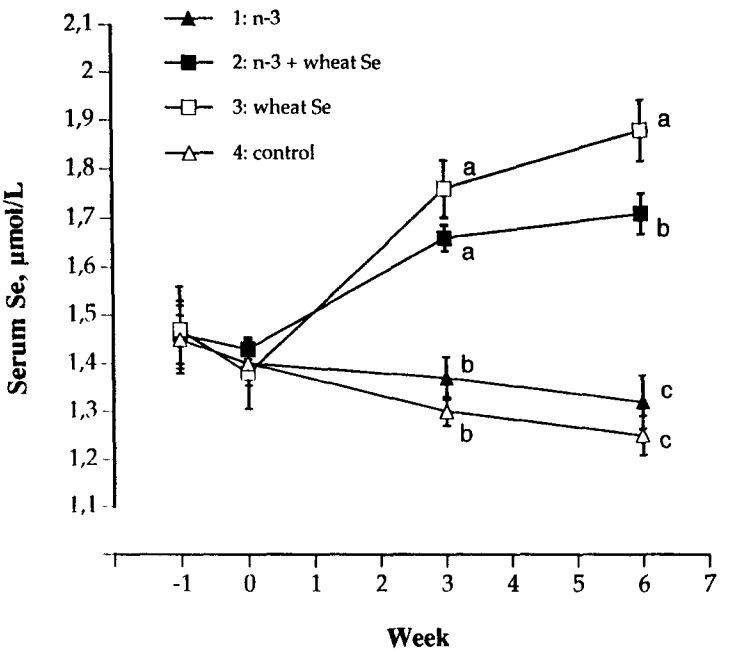

Fig. 1. Time-course of serum Se concentrations in group 1 (*n*-3 + placebo bread), group 2 (*n*-3 + wheat Se), group 3 (placebo capsules + wheat Se), and group 4 (placebo capsules + placebo bread). Values with different letters at a given time-point are significantly different ($p < 0.05$, Duncan's multiple-range test). Mean ± SE.

Table 4
Changes in Whole-Blood Se, Plasma Vitamin C, Plasma Vitamin E, Plasma TBARS, and Conjugated Dienes During 12-wk Supplementation with Wheat Se or Placebo Bread and *n*-3 PUFA[1,2,3]

					2-way ANOVA[4]		
	Group 1	Group 2	Group 3	Group 4	n-3 PUFA	Se	n-3 x Se
	% change				p-value		
Whole blood Se	- 4.6 ± 9.2[a]	20.2 ± 7.8[c]	36.9 ± 9.0[b]	10.8 ± 9.0[a]	0.12	≤ 0.0004	0.02
Plasma vitamin C	- 3.9 ± 20.8[a]	1.9 ± 12.0[a]	2.4 ± 27.8[a]	- 0.8 ± 10.5[a]	0.80	0.52	0.86
Plasma vitamin E[5]	0.7 ± 6.3[a]	- 0.4 ± 7.6[a]	0.5 ± 3.9[a]	2.0 ± 5.3[a]	0.32	0.63	0.42
Conjugated dienes	130.3 ± 74.0[b]	105.8 ± 94.6[ab]	61.8 ± 59.1[a]	- 12.5 ± 70.6[a]	0.003	0.78	0.03
TBARS	126.3 ± 79.0[b]	56.6 ± 64.9[a]	3.5 ± 11.5[a]	- 4.7 ± 46.5[a]	≤ 0.0004	0.03	0.015

[1]Values are mean percentage change ± SD for 6–8 persons.
[2]The significance of difference between any two groups was tested by Duncan's multiple-range test. Data in the same row with the same superscript letter are not significantly different.
[3]All changes are adjusted for initial values.
[4]Analysed by two-way ANOVA, each value represents a *p*-value.
[5]Plasma vitamin E/mg plasma cholesterol.

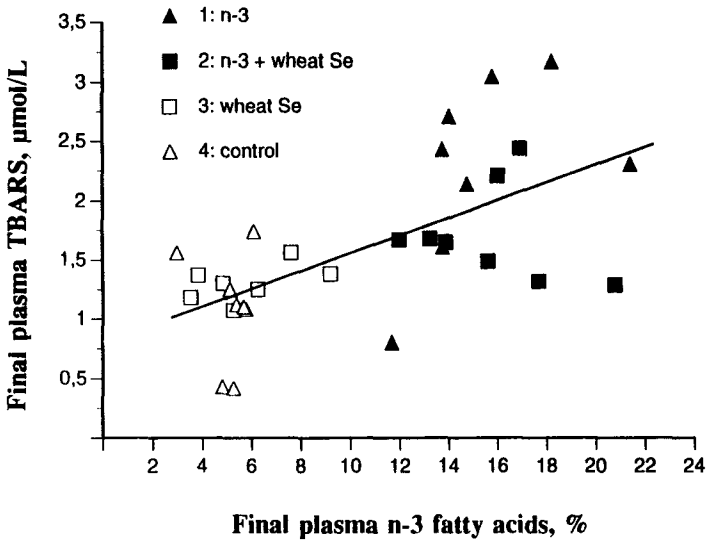

Fig. 2. Final plasma TBARS vs final plasma *n*-3 fatty acids, $r = 0.53$, $p < 0.001$, $n = 31$ ($f[x] = 7.52\ 10^{-2}x + 8.03\ 10^{-1}$). Correlation between changes in the variables: $r = 0.65$, $p < 0.001$. The correlation between final plasma conjugate dienes and *n*-3 PUFA was 0.57, $p < 0.001$.

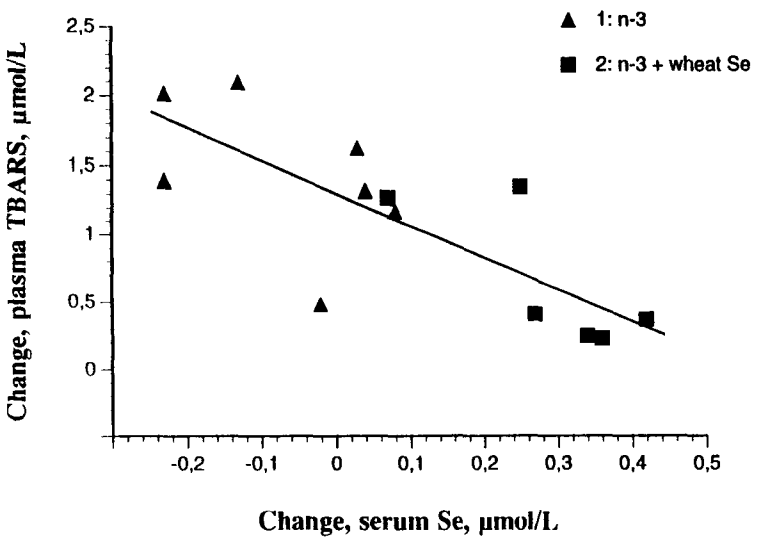

Fig. 3. Changes in plasma TBARS (adjusted for initial concentrations) vs changes in serum Se in the *n*-3 supplemented groups: $r = -0.78$, $p < 0.002$, $n = 13$ ($f[x] = -2.35x + 1.30$). The corresponding correlation with changes in conjugated dienes and serum Se was $r = -0.60$, $p < 0.03$, $n = 14$. Within the Se-supplemented group (group 2), this correlation was $r = -0.90$, $p < 0.006$, $n = 7$.

DISCUSSION

This study has shown that there is a relationship between the metabolism of Se and fatty acids. Supplemental *n*-3 fatty acids reduced the increases in serum Se concentrations achieved by wheat Se ingestion. Furthermore, there were significant interactions between Se and *n*-3 fatty acids, the net result being that *n*-3-induced increases in TBARS and conjugated dienes were reduced by Se supplementation.

The participating subjects would by most standards be evaluated to be Se-replete. Norwegians have, on the average, the highest serum Se concentrations in Europe (1.3–1.5 μmol/L) *(31)*. We have shown earlier that at this high level, blood, serum, and platelet GSH-Px are all fully expressed with respect to Se, and do not respond to Se supplementation *(26,32)*. The extent of inducibility of GSH-Px is not known, but we found that neither serum nor platelet GSH-Px rose in response to a high intake of fat fish (250 g/d), providing 2.6 g *n*-3 fatty acids/d and 115 μg Se/d *(31)*. Thus, we decided not to measure GSH-Px in the present study.

The daily dietary α-tocopherol intake of 5.8 mg would probably place the total intake of tocopherol equivalents close to the 8 mg/d recommended by US NRC *(33)*. Furthermore, the tocopherol intake must be seen in connection with the low-fat content of the diet of the female participants, only 24 ± 5 energy % coming from fat. The α-tocopherol:PUFA ratio of the diet was 0.5, on the borderline of what is considered to be adequate *(34,35)*, but none of the subjects had, initially, lowered plasma α-tocopherol concentrations.

The ratio α-tocopherol:PUFA was 0.5 in the *n*-3 fatty acid capsules, providing a supplemental α-tocopherol intake of 3.6 mg/d. One might speculate whether a higher α-tocopherol intake could have influenced the results, but we obtained no indications of this. When changes in tocopherol per mg cholesterol were adjusted for initial concentrations, TBARS and conjugated dienes were not significant explanatory variables in multiple regression models for tocopherol changes.

The duplicate portion method employed to analyze the dietary intake of Se, α-tocopherol, and PUFA has been criticized because of a tendency to underreport food intake *(36,37)*. We believe our group of nutrition students to be especially motivated and, therefore, correct about their reportings, as illustrated by the good correlation between dietary protein and urinary nitrogen (N) (r = 0.73, p<0.001). There was also a very good correspondence among intakes of total fat, saturated fats, and monounsaturated fats calculated from written food records and analyzed from duplicate portions (r = 0.92, 0.85, and 0.75, respectively, p < 0.001, in all three cases). Nevertheless, the actual intakes of these nutrients in the intervention period may have been somewhat higher.

The PUFA doses used in this study are comparable to those used in a number of other studies *(34,38,39)*. Diet, age, and sex of the subjects seem to influence the response to such supplementation *(12,21)*. In a

recent study comprising women from the same age group, but using lower doses of *n*-3 fatty acids, plasma fatty acid changes similar to ours (but smaller) were observed *(40)*.

Long-chain PUFA are highly susceptible to lipid peroxidation. At high intakes, they might therefore increase in vivo peroxidative stress, even when plasma concentrations of antioxidants are maintained at a normal level. This study is an example of this: in spite of no change in the plasma ratio of α-tocopherol:mg cholesterol, conjugated dienes rose by 130% and TBARS by 126% after 6 wk of *n*-3 PUFA supplementation in group 1 (*n*-3 + placebo bread) (Table 4).

A number of different methods for measurement of lipid peroxidation have been described. These tests measure compounds formed at different stages in the peroxidation process, and malondialdehyde, as measured by TBARS, is only one of several peroxidation products. Thus, if possible, more than one method should be used *(41)*. Under our experimental conditions, formations of conjugated dienes and TBARS were strongly correlated ($r = 0.76$, $p < 0.0005$), indicating that TBARS formation, as measured, was a good indicator of the degree of lipid peroxidation.

Serum Se concentrations were reduced in the control group (Fig. 1), but in spite of a lower intake of Se, serum Se did not change significantly in group 1 (*n*-3 + placebo bread) (Table 2 and Fig. 1). This reduction in serum Se should be ascribed to the substitution of medium-Se commercial flour with low-Se Norwegian-grown flour in the placebo bread. Thus, somewhat unexpectedly, we could not observe any Se-lowering effect of *n*-3 PUFA added to a normal diet. This is in contrast to the results from a study in Italy, where increased intake of PUFA gave a significant reduction in serum Se *(13)*. In that study, however, initial serum Se concentrations were just above half of ours, i.e., 0.83 vs 1.40 μmol/L. At such low blood concentrations, GSH-Px is usually far from fully expressed with respect to Se, and increased enzyme turnover could more directly lead to decreases in serum Se concentrations. In our subjects, GSH-Px turnover is probably only a minor pathway of Se loss. Figure 3, showing the relationship between serum Se changes and plasma TBARS changes, indicates some kind of turnover effect.

It was also somewhat unexpected that plasma α-tocopherol levels only underwent marginal changes, in spite of a substantial decrease ($p < 0.05$) in the plasma ratio of α-tocopherol:*n*-3 PUFA in group 1 (*n*-3 PUFA + placebo bread). This might indicate that α-tocopherol was effectively regenerated under the prevailing conditions, but it could also indicate that the supplemental α-tocopherol was just enough to maintain plasma concentrations. In the latter case, supplementation regimens leading to higher α-tocopherol in plasma and tissues might decrease the PUFA-induced lipid peroxidation.

n-3 PUFA supplementation influenced the response of serum Se to increased Se intake (Fig. 1). Moreover, as Fig. 3 shows, there are strong indications that this is directly related to lipid peroxidation. In a well-

fitting multiple regression model ($r^2 = 0.70$, $p < 0.003$), plasma total *n*-3 fatty acids and plasma conjugated dienes were the most important factors influencing the serum Se response in the Se-supplemented individuals (groups 2 and 3).

Two-way ANOVA of the changes in TBARS and conjugated dienes indicate the existence of interaction effects of *n*-3 PUFA and Se (Table 4). Moreover, there was an effect of Se on TBARS independent of PUFA. Because PUFA are the main determinants of peroxidation, the results must be interpreted as Se supplementation modifying the peroxidative effects of PUFA.

The mechanism of this interaction is not known. In retrospect, we regret that we did not measure GSH-Px, but if the effects were largely mediated through this enzyme, one should expect a reduction in serum Se in the PUFA + placebo bread group (group 1). Since GSH-Px reduces fatty acid hydroperoxides, with no effect on the endoperoxides from which TBARS are formed, one might also expect supplementation to influence the relationship between conjugated dienes and TBARS. The relationships between concentrations of and changes in plasma TBARS and conjugated dienes were, however, apparently unaffected by Se supplementation. This points to functional dependencies between different parts of the antioxidant defense system, but it also puts the role of GSH-Px further into question.

Some other selenoproteins might be involved in the interaction. Burk and Hill have suggested an antioxidant role for selenoprotein P *(42)*. Since GSH-Px has a long half-life when the antioxidant defense system is not perturbed, the data could indicate the presence of an Se compound having a function similar to, but not identical with, GSH-Px. To a much higher degree than GSH-Px, this compound could be metabolized during its interaction with peroxidation promoters. In animal studies, Burk et al. observed that Se supplementation could inhibit diquat and paraquat-induced free radical generation before GSH-Px activities had risen *(43)*. This phenomenon may be related to our observations. The existence of such a compound would also explain the findings of Willett et al. *(44)* and Lane et al. *(45)*. Willett et al. found that differences in serum Se above the level where GSH-Px is usually saturated were predictive for risk of cancer, and Lane et al. found reduced blood Se in oil industry workers, the reduced concentrations still being above those normally associated with GSH-Px saturation. In light of the present findings, GSH-Px turnover is probably only partially responsible for the reduced blood or serum Se concentrations in group 2 (*n*-3 + wheat Se).

Further studies of the Se–PUFA interactions reported here should probably assess a number of Se-related parameters in addition to serum Se. Platelet and serum GSH-Px should be measured, and platelet Se whenever possible, since the platelet Se response to wheat Se supplementation in an earlier study (no PUFA given) was dependent on platelet arachidonic acid, but not on platelet GSH-Px (Meltzer, unpublished

observations). In addition, selenoprotein P and possibly other selenoproteins should be monitored.

Under the present study conditions, the conjugated dienes, mostly resonance stabilized radicals from *n*-3 PUFA, seem to a be good indicator of in vivo fatty acid-induced peroxidation processes. There is, however, a need for more information about different peroxidation measures and their interrelations. In multiple regression models, plasma *n*-3 PUFA concentrations and dietary arachidonic acid (20:4 *n*-6) were the main determinants of plasma TBARS and conjugated dienes, when all groups were taken together (*see also* Fig. 3 and legend). Within group 2 (*n*-3 + wheat Se), however, there was in fact a negative correlation between changes in plasma EPA and conjugated dienes ($r = -0.72$, $p < 0.05$, $n = 8$). There was a very strong negative correlation between changes in plasma conjugated dienes and changes in serum Se in this group ($r = -0.90$, $p < 0.006$, $n = 7$). Taken together, the observations might indicate that *n*-3 fatty acids are better protected in the presence of Se of wheat origin.

The study was not designed to address the question whether dietary Se may affect *n*-3 fatty acid concentrations in the body at normal PUFA intakes, but we obtained an indication of an effect of wheat Se on the eicosanoid potential: the plasma eicosanoid potential rose significantly in group 3 (placebo capsule + wheat Se) as compared to group 4 (all placebo), in spite of a lower EPA intake in this group. This may be indicative of an EPA-sparing effect of Se supplementation.

In conclusion, our study demonstrates interactions between *n*-3 fatty acids and Se affecting the plasma concentrations of lipid peroxidation products. However, which Se compounds are involved is not known.

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